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L3: Entry 15 of 20 File: USPT Jul 13, 2004

US-PAT-NO: 6762280
DOCUMENT-IDENTIFIER: US 6762280 B2

TITLE: High throughput assays for the proteotytic activities of clostridial neurotoxins

DATE-ISSUED: July 13, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
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US-CL-CURRENT: 530/300; 435/183, 435/252.4, 435/4, 435/7.1, 435/7.71, 435/7.72, 435/842, 530/324, 530/326, 530/333, 530/335, 530/337, 530/344, 530/345, 530/350, 930/10, 930/20

CLAIMS:

What is claimed is:

1. A botulinum neurotoxin serotype A substrate containing a fluorescent signal moiety on one side of the cleavage site that produces a fluorescent signal and a moiety that quenches the magnitude of said signal on the other side of the cleavage site, wherein when the substrate is cleaved, an increase in fluorescence is produced, and wherein said substrate is a peptide identified as SEQ ID NO: 1 or SEQ ID NO:2.

2. A method for detecting the presence of botulinum neurotoxin serotype A proteolytic activity in a sample, said method comprising: a) mixing the sample with the peptide substrate according to claim 1, and b) detecting an increase in fluorescent signal produced from proteolytic cleavage of said substrate.

3. A method for measuring the concentration of botulinum neurotoxin serotype A in a sample, said method comprising: a) mixing the sample with the peptide substrate according to claim 1, b) measuring an increase in fluorescent signal with time produced from proteolytic cleavage of said substrate, and c) determining the concentration of said neurotoxin by correlation to a standard of said neurotoxin.

4. A kit for determining the concentration of botulinum neurotoxin serotype A in a sample, the kit containing in close confinement; (i) one or both peptide substrates according to claim 1 cleavable by said botulinum neurotoxin; and (ii) said botulinum neurotoxin standard.

5. A botulinum neurotoxin serotype A substrate comprising a peptide or protein which is optionally immobilized to a solid material and which contains a fluorescent moiety that produces a measurable fluorescent signal, wherein when the substrate is cleaved, the fluorescent signal is released, and wherein said

substrate is a peptide identified as SEQ ID NO:8 or SEQ ID NO:11.

6. A method for detecting the presence of botulinum neurotoxin serotype A proteolytic activity in a sample, said method comprising: a) mixing the sample with the peptide substrate according to claim 5, and b) detecting an increase in fluorescent signal produced from proteolytic cleavage of said substrate.

7. A method for measuring the concentration of botulinum neurotoxin serotype A in a sample, said method comprising: a) mixing the sample with the peptide substrate according to claim 5, b) measuring an increase in fluorescent signal with time produced from proteolytic cleavage of said substrate, and c) determining the concentration of said neurotoxin by correlation to a standard of said neurotoxin.

8. A kit for determining the concentration of botulinum neurotoxin serotype A in a sample, the kit containing in close confinement; (i) one or both peptide substrates according to claim 5 cleavable by said botulinum neurotoxin; and (ii) said botulinum neurotoxin standard.

9. A method for identifying a compound that inhibits or enhances the proteolytic activity of botulinum neurotoxin serotype A, said method comprising: a) preincubating the neurotoxin with a test compound to make a neurotoxin-compound solution, b) exposing said solution to the substrate according to claim 5, c) measuring fluorescent signal resulting from proteolytic cleavage of said substrate by said neurotoxin, and d) comparing said fluorescent signal from the solution of step a) with a control, wherein the control is the solution of step a) in the absence of the test compound, and wherein an increase in fluorescent signal indicates a compound that enhances neurotoxin activity and a decrease in fluorescent signal indicates a compound that inhibits said neurotoxin.

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L3: Entry 10 of 20 File: PGPB Apr 24, 2003

PGPUB-DOCUMENT-NUMBER: 20030077685
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030077685 A1

TITLE: High throughput assays for the proteotytic activities of clostridial neurotoxins

PUBLICATION-DATE: April 24, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Stafford, Robert G.	Ranson	WV	US	

US-CL-CURRENT: 435/23; 435/34, 530/350

CLAIMS:

What is claimed is:

1. A clostridial neurotoxin substrate comprising any peptide or protein that can serve as a substrate for the proteolytic activity of any clostridial neurotoxin, said protein or peptide having been modified to contain a signal moiety on one side of the cleavage site, and a moiety on the other side of the cleavage site that quenches the magnitude of that signal such that when the substrate is cleaved, an increase in signal is produced.

2. The substrate according to claim 1 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A.

3. The substrate according to claim 2 wherein said substrate is a peptide identified in SEQ ID NO: 1 or SEQ ID NO: 2.

4. The substrate according to claim 1 wherein said clostridial neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

5. The substrate of claim 4 wherein said substrate is identified in SEQ ID NO: 3 and SEQ ID NO: 4.

6. The substrate according to claim 1 wherein said clostridial neurotoxin is botulinum neurotoxin serotype D or botulinum neurotoxin serotype F.

7. The substrate of to claim 6 wherein said substrate is chosen from the group consisting of a peptide identified in SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

8. A method for detecting the presence of clostridial neurotoxin proteolytic acitivity in a sample said method comprising mixing the sample with a peptide substrate according to claim 1, and detecting an increase in signal produced from proteolytic cleavage of said substrate.

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9. A method for measuring concentration of neurotoxin in a sample, comprising mixing the sample with a peptide substrate according to claim 1, measuring an increase in signal with time produced from proteolytic cleavage of said substrate and, determining the concentration of said neurotoxin by correlation to a standard.

10. The method according to claim 9 wherein said neurotoxin is botulinum neurotoxin serotype A.

11. The method according to claim 10 wherein said peptide substrate is a peptide identified in SEQ ID NO: 1 or SEQ ID NO: 2.

12. The method according to claim 9 wherein said neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

13. The method according to claim 12 wherein said peptide substrate is a peptide identified in SEQ ID NO: 3 or SEQ ID NO: 4.

14. The method according to claim 9 wherein said neurotoxin is botulinum neurotoxin serotype D or F.

15. The method according to claim 14 wherein said peptide substrate is chosen from the group consisting of a peptide identified in SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

16. A kit for determining the concentration of a clostridial neurotoxin in a sample, the kit containing in close confinement, (i) one or more peptide substrates according to claim 1 cleavable by said clostridial neurotoxin; (ii) said clostridial neurotoxin standard.

17. The kit according to claim 16 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A and the peptide substrate is one or both of the peptides identified in SEQ ID NO: 1 and SEQ ID NO: 2.

18. The kit according to claim 16 wherein said clostridial neurotoxin is botulinum neurotoxin serotype B or tetanus toxin and the peptide substrate is one or both of the peptides identified in SEQ ID NO: 3 and SEQ ID NO: 4.

19. The kit according to claim 16 wherein said clostridial neurotoxin is botulinum neurotoxin serotype D or F and the peptide substrate is one or more of the peptides identified in SEQ ID NO: 5, SEQ ID NO: 6, and SEQ ID NO: 7.

20. A botulinum neurotoxin substrate comprising any peptide or protein that can serve as a substrate for the proteolytic activity of any clostridial neurotoxin, said protein or peptide having been modified so that it can be attached on one side of the proteolytic cleavage site to a solid material.

21. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A.

22. The substrate according to claim 21 wherein said substrate is a peptide identified in SEQ ID NO: 8 or SEQ ID NO: 11.

23. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

24. The substrate of claim 23 wherein said substrate is identified in SEQ ID NO: 9.

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25. The substrate according to claim 20 wherein said clostridial neurotoxin is botulinum neurotoxin serotype D or serotype F.

26. The substrate of claim 25 wherein said substrate is a peptide identified in SEQ ID NO: 10.

27. The substrate of claim 20 wherein said botulinum neurotoxin is botulinum neurotoxin serotype E.

28. The substrate of claim 27 wherein said substrate is a peptide identified in SEQ ID NO: 11 and 12.

29. A method for detecting the presence of clostridial neurotoxin proteolytic acitivity in a sample said method comprising mixing the sample with a peptide substrate according to claim 20, and detecting an increase in signal produced from proteolytic cleavage of said substrate.

30. A method for measuring concentration of neurotoxin in a sample, comprising mixing the sample with a peptide substrate according to claim 20, measuring an increase in signal with time produced from proteolytic cleavage of said substrate and, determining the concentration of said neurotoxin by correlation to a standard.

31. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype A.

32. The method according to claim 31 wherein said peptide substrate is a peptide identified in SEQ ID NO: 8 or SEQ ID NO: 11.

33. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype B or tetanus toxin.

34. The method according to claim 33 wherein said peptide substrate is a peptide identified in SEQ ID NO: 9.

35. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype D or F.

36. The method according to claim 35 wherein said peptide substrate is a peptide identified in SEQ ID NO: 10.

37. The method according to claim 30 wherein said neurotoxin is botulinum neurotoxin serotype E.

38. The method according to claim 37 wherein said peptide substrate is a peptide identified in SEQ ID NO: 11 or SEQ ID NO: 12.

39. A kit for determining the concentration of a clostridial neurotoxin in a sample, the kit containing in close confinement, (i) one or more peptide substrates according to claim 20 cleavable by said clostridial neurotoxin; (ii) said clostridial neurotoxin standard.

40. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum neurotoxin serotype A and the peptide substrate is one or both of the peptides identified in SEQ ID NO: 8 and SEQ ID No: 11.

41. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum

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neurotoxin serotype B or tetanus toxin and the peptide substrate is a peptide identified in SEQ ID NO: 9.

42. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum neurotoxin serotype D or F and the peptide substrate is a peptide identified in SEQ ID NO: 10.

43. The kit according to claim 39 wherein said clostridial neurotoxin is botulinum neurotoxin serotype E and the peptide substrate is one or more of the peptides identified in SEQ ID NO: 11 or SEQ ID NO: 12.

44. A method for identifying inhibitors or enhancers of proteolytic activity of a clostridial neurotoxin comprising: preincubating a neurotoxin with a test compound to make a neurotoxin-compound solution, exposing said solution to a substrate of said neurotoxin according to claim 20, measuring signal resulting from the proteolysis of said substrate by said neurotoxin, and comparing said signal with controls, wherein an increase in signal indicates a compound which enhances neurotoxin activity and a decrease in signal indicates a compound with inhibits said neurotoxin.

45. The method according to claim 44 wherein said neurotoxin is botulinum neurotoxin serotype A and the substrate is a peptide identified in SEQ ID NO: 8 or SEQ ID NO: 11.

46. The method according to claim 44 wherein said neurotoxin is botulinum neurotoxin serotype B or tetanus toxin and the substrate is a peptide identified in SEQ ID NO: 9.

47. The method according to claim 44 wherein said neurotoxin is botulinum neurotoxin serotype D or F and the substrate is a peptide identified in SEQ ID NO: 10.

48. The method according to claim 44 wherein said neurotoxin is botulinum neurotoxin serotype E and the substrate is one or more of the peptides identified in SEQ ID NO: 11 or SEQ ID NO: 12.

49. A method for identifying a serotype of a clostridial neurotoxin in a sample suspected of containing a neurotoxin, the method comprising incubating the sample with antibodies against each clostridial neurotoxin such that a neurotoxin is bound to its serotype-specific antibody, removing unbound components, adding activation solution such that clostridial protease is activated, adding solutions containing clostridial neurotoxin peptide substrates according to claim 1 to said activated protease, detecting signal generated from proteolysis of said substrate by said protease, wherein a signal above control indicates presence of a neurotoxin, and determining the serotype of the clostridial neurotoxin by noting the specificity of the antibody.

50. The method of claim 49 wherein the antibodies are bound to a solid material.

51. The method of claim 50 wherein the solid material is a multiwell plate.

52. The method of claim 51 wherein each well contains an antibody specific for a different neurotoxin serotype.

53. The method of claim 49 wherein each peptide substrate is labeled with a different signal.

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54. A kit for identifying a serotype of a clostridial neurotoxin in a sample suspected of containing a neurotoxin, comprising serotype-specific antibodies clostridial neurotoxin standards, and peptide substrates according to claim 1.

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